

PATENT
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Colleen Coyne

Colleen Coyne

Printed name of person mailing correspondence

Signature of person mailing correspondence

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	David Moore et al.	Art Unit:	1646
Serial No.:	09/365,576	Examiner:	M. Pak
Filed:	August 2, 1999	Customer No.:	21559
Title:	RETINOID X RECEPTOR-INTERACTING POLYPEPTIDES AND RELATED MOLECULES AND METHODS		

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF DR. DAVID MOORE UNDER 37 C.F.R. § 1.131

I, David Moore, declare that:

1. I am an inventor of the invention described and claimed in the above-identified patent application.
2. The present claims of the application recite retinoid X receptor-interacting proteins that have an amino acid sequence that is at least 85% identical to that of RIP15.
3. The other inventors and I conceived of, and reduced to practice, the claimed subject matter of the present application prior to November 10, 1993.
4. The reduction to practice of the claimed invention is evidenced by Exhibits 1 and 2 annexed hereto. The dates of Exhibits 1 and 2 have been redacted in accordance with the standard practice, but are all prior to November 10, 1993. The experiments described and summarized in Exhibits 1 and 2 were performed by Wongi Seol, another inventor of the claimed invention. These experiments were carried out in the United States prior to November 10, 1993.

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5. As described in the present application, we performed an *in vivo* interaction trap assay to isolate cDNA molecules encoding proteins that interact with the retinoid X receptor (RXR). For this assay, a mouse cDNA library was introduced into yeast that express a LexA-RXR fusion protein and that contain β -galactosidase and LEU2 genes under the control of LexA binding sites. LexA-RXR is not a strong transcriptional activator in yeast. However, LexA-RXR activates expression from LexA binding sites in cells which also express a fusion protein consisting of a transcriptional activation domain joined to another protein which interacts specifically with RXR (as described on pages 12 and 25-27 of the specification).

Clone 15 which encodes RIP15 was isolated in this assay based on its ability to induce expression of both β -galactosidase and LEU2, indicating that it encoded a protein that interacted with RXR.

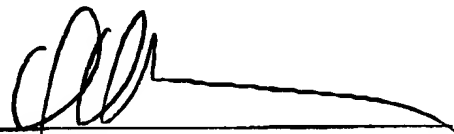
6. Exhibit 1 is a laboratory notebook page that contains a picture of an agarose gel showing the analysis of the plasmid isolated from selected yeast clone 15 and transferred into *E. coli* for analysis (lower panel). The plasmid was purified from *E. coli* and digested with restriction enzymes to determine the size of the cDNA insert in the plasmid. Based on the migration of the restriction enzyme-digested cleavage product in the agarose gel, the cDNA insert was approximately 4.0 kDa (lower left corner of Exhibit 1). A region from this cDNA insert was used as a hybridization probe to isolate the full-length RIP15 coding sequence from a mouse cDNA library, as described on page 27 of the specification.

7. This full-length RIP15 cDNA was sequenced prior to November 10, 1993. Exhibit 2 contains exemplary RIP15 cDNA sequence. In particular, the sequence of primer ip15 PCR 42mer¹ which is listed in Exhibit 2 was designed based on the 5' terminus of this full-length RIP15 cDNA sequence. This primer was used to amplify the RIP15 sequence and add restriction sites flanking the RIP15 sequence to facilitate subsequent subcloning into an expression vector for the production of RIP15 protein. In this primer, the first nucleotide of the second RIP15 codon was altered to generate an NcoI restriction site and Kozak consensus nucleotides were included upstream of the start iAUGi codon to maximize expression of RIP15 protein. The nucleotides from the RIP15 cDNA sequence that were included in this primer are 5'-CTTCCCCCACAAGTTCTCTG-3'.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are

punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: 12/21/01



David Moore, Ph.D.
Professor
Molecular & Cellular Biology
Baylor College of Medicine

Brian :

Exhibit 2

Please make these oligomers. Thanks.

Wongi

p14 PCR 36mer

5'

GCG CGC AAG CTT GCC ACC ATG GCC GCG GCA TCG
Hind III Not I

GCA

p15 PCR 42mer

5' GCG CGC AAG CTT GCC ACC ATG ~~GCT~~ TCC CCC ACA AGT
Hind III

TCT ~~CTG~~ CTG